

# THE CYSTINE CONTENT OF ELEVEN VARIETIES OF SOYBEANS<sup>1</sup>

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## INTRODUCTION

The soybean (*Soja max* (L.) Piper) is fast becoming a factor of major importance in American agriculture. A valuable crop to the agronomist, a source of raw materials for many industrial commodities, such as glue, plastics, paint, artificial wood, etc., as well as a valuable feed and food supplement for both man and animal, the soybean occupies an important place among the cultivated crops in many parts of the world. While soybean hay, whole soybeans, and soybean oil are valuable foodstuffs, the proteins of the beans are of chief interest and importance so far as the nutritive value of the crop is concerned.

By 1925, 1,133 varieties had been described, and of these more than 100 named varieties are widely grown or are being distributed in the United States (16).<sup>2</sup> Some varieties are useful for their oil content or for their feeding value for animals. However, a number of the newer varieties are valuable as food for human beings. Woodruff (28) of the University of Illinois has tested 467 varieties of edible soybeans for their eating qualities and has rated 6 as very good and 70 as good.

The value of soybeans or soybean oil meal, or of any feed, as a source of protein to the animal body depends not only upon the total amount of protein present, but also upon its digestibility and biological value when consumed. That the total amount of protein in a sample of soybeans may vary widely, depending upon the variety, soil fertility, climatic conditions, etc., has been shown by a number of investigations. References to investigations of this nature to 1938 may be found in a review by the Soybean Nutritional Research Council (10).

Osborne and Campbell (19) isolated several types of proteins from soybeans and gave to the principal one, a globulin, the name glycinin. This protein amounted to 16.6 percent of the meal or between 80 and 90 percent of the total protein present. They also obtained evidence of the existence of another globulin which was even more soluble than glycinin. In addition to these two globulins there were isolated a legumelin, amounting to about 1.5 percent of the seed, and a small amount of protease.

A number of investigations (9, 12, 14, 15) have indicated that the proteins of raw (usually defatted) soybeans have a low nutritive value. Mitchell and Smuts (15) and, later, Shrewsbury and Bratzler (22), Hayward, Steenbock, and Bohstedt (8), and Smuts and Marais (23) have shown that the amino acid limiting the utilization of the absorbed nitrogen from soybeans was cystine.

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<sup>2</sup> Italic numbers in parentheses refer to Literature Cited, p. 212.

In view of these findings and the wide variations in the protein content of different varieties of soybeans, it is of interest to examine, in more detail, the chemical nature and, in particular, the cystine content of different varieties of soybeans. Unfortunately there has been but little work done on the methionine content of these beans.

Osborne and Clapp (20) analyzed glycinin and found the amino acid content to be similar to that of casein. They reported, however, no cystine values for the isolated protein. Csonka and Jones (4) also analyzed the glycinin from the seeds of several varieties of soybeans for cystine, tryptophane, and tyrosine. These workers reported a variation in the cystine content between 0.74 percent for the Illini variety and 1.45 percent for the Manchu variety. Csonka, Murphy, and Jones (3) presented evidence which indicated that glycinin, as ordinarily prepared, was not an individual protein and Csonka and Jones explained the wide variation in the cystine content of their different glycinin preparations on the basis of variations in the relative proportions in which the different globulins comprising the glycinin fractions were present in the different varieties of soybeans.

Later (5), Csonka and Jones, after failing to obtain satisfactory cystine determinations on defatted soybean meal, determined the cystine content of 10-percent sodium chloride extracts (containing 85 to 90 percent of the total nitrogen of the meal) of six varieties of soybeans. At the same time tryptophane and tyrosine were determined directly on the defatted meals of the same varieties. Using a modification (2) of the Sullivan (24) method, they found variations in the cystine content of the defatted meals of from 0.287 percent for the Illini variety to 0.491 percent for a Herman variety.

Sasaki (21) analyzed soybean protein for several amino acids but reported no values for either of the sulfur-containing amino acids. Mashino and Nishimura (13) studied the nitrogen distribution, by the Van Slyke method, in the defatted beans from two varieties, Tsurunoko-daizu and Machurian, the latter of which was analyzed in the unheated and heated states after the oil had been removed either with solvent or by pressure. They found but little variation in the nitrogen distribution of their samples and reported an average of 1.74 percent of cystine nitrogen. Earlier, and also using the Van Slyke method, Hamilton, Uyei, Baker, and Grindley (7) reported an average value of 1.46 percent in Medium Early Yellow soybeans, and Nollau (17) reported 1.52 percent, both values being cystine nitrogen expressed as a percent of the total nitrogen.

Baernstein (1) found, in his study of a number of proteins, that soybean glycinin contained 1.84 percent methionine. The methionine sulfur amounted to 47.2 percent of the total sulfur. However, Tomiyama and Hanada (25) were able to isolate but 0.08 percent of methionine from dry, ash-free soybean protein.

The study to be reported here is concerned with the cystine content of 11 varieties of soybeans, all grown in the same year (1933) on a small plot of ground, the soil of which was very uniform. The beans were taken from groups of plants systematically replicated 10 times over the entire plot.<sup>3</sup>

<sup>3</sup> These samples were furnished by Prof. C. M. Woodworth of the Department of Agronomy.

## PROCEDURE

Each of the 11 varieties of soybeans used was clean and well cured when received. The beans were first ground, avoiding the generation of heat, so as to pass through a 40-mesh sieve. In a few cases the beans were ground even finer. The method of preparation of a fat-free, carbohydrate-free protein sample was similar to that used by Hamilton, Nevens, and Grindley (6), with only slight modifications. In this procedure triplicate 15-gm. samples of the finely ground, dry beans were thoroughly extracted, first with absolute ethyl ether, then with absolute ethyl alcohol. These extracts were discarded after they were found to be practically free of nitrogen.

The residues from the ether and alcohol extractions were then extracted with dilute sodium hydroxide solutions. Eight to ten extractions, using 400 cc. of 0.1-percent Sodium hydroxide for each extraction, were first made. Then extractions were made in which 200 cc. of 1-percent sodium hydroxide was used for each extraction. These extractions were continued until the extract no longer gave a test for protein, usually 6 to 8 extractions being necessary. Centrifuging and decanting were used to separate extract from residue. Each extract was immediately neutralized with acetic acid after being decanted.

The combined extracts from each sample were then carefully concentrated to about 200 cc. An equal volume of concentrated hydrochloric acid was added and the protein hydrolyzed by gentle boiling under a reflux condenser for about 20 hours. The hydrolyzate was then cautiously concentrated in a beaker to about 125 to 150 cc. and neutralized with 40-percent potassium hydroxide solution. After the solution was made barely acid to litmus, 2 to 3 gm. of norit were added, and the mixture boiled gently for a few minutes. The norit was filtered off and washed with hot water. After the filtrate had stood overnight, it was again treated with a small amount of norit. The solution thus obtained was clear and slightly yellowish, but this color did not seem to interfere with the colorimetric estimation of cystine.

Cystine was determined in the extracts by Lugg's (11) modification of the Sullivan (24) method.

## RESULTS

The results of this study are presented in the accompanying tables. Table 1 gives the usual chemical analyses and table 2 gives the results of the cystine determinations on the 11 varieties of soybeans. The routine chemical examination shows a considerable varietal variation between the samples, this being particularly true of the protein content. The percentage of protein (total nitrogen  $\times$  6.25) varied from 35.88 (Peking) to 43.69 (Manchu), averaging 39.11 for the 11 varieties. The ether extract varied from 16.07 (Ito San) to 19.16 (Mansoy), averaging 17.86 percent for all varieties. Since these varieties had about the same moisture content and since all were grown in the same year on uniform soil in a small plot of ground, the differences observed strongly indicate varietal differences of considerable magnitude.

TABLE 1.—Percentage composition of 11 varieties of soybeans grown in Illinois and harvested in 1933

Variety	Moisture	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract	Ash	Total nitrogen
Illini.....	7.06	37.75	18.82	4.37	27.28	4.72	6.04
Ohio 13-177.....	7.04	38.31	18.75	4.54	26.32	5.04	6.13
Harbinsoy.....	7.12	39.69	18.45	4.24	25.50	5.00	6.35
Virginia.....	6.89	38.81	17.90	4.65	26.71	5.04	6.21
Mansoy.....	7.17	36.25	19.16	4.41	27.84	5.17	5.80
Ito San.....	6.68	41.44	16.07	3.97	26.74	5.10	6.63
Dunfield.....	6.73	36.38	17.62	4.12	30.45	4.70	5.82
Manchu.....	7.34	43.69	17.80	3.69	22.44	5.04	6.99
Peking.....	7.20	35.88	17.38	5.03	29.41	5.10	5.74
Mandarin.....	7.82	41.44	16.66	4.12	24.92	5.04	6.63
Morse.....	7.41	40.56	17.91	3.97	25.02	5.13	6.49
Average.....	7.13	39.11	17.86	4.28	26.61	5.01	6.26

TABLE 2.—Cystine content of 11 varieties of soybeans

Variety	Total nitrogen extracted	Cystine content		
		In whole soybeans <sup>1</sup>	Cystine nitrogen as percent of total nitrogen	Cystine per gram of nitrogen
	Percent	Percent	Percent	Milligrams
Illini.....	96.6	0.366	0.696	59.7
Ohio 13-177.....	99.1	.266	.433	42.3
Harbinsoy <sup>2</sup> .....	92.8	.256	.464	39.8
Virginia.....	96.5	.213	.385	33.1
Mansoy.....	95.5	.553	1.042	89.4
Ito San.....	90.8	.413	.703	60.3
Dunfield.....	97.9	.439	.858	73.7
Manchu.....	99.4	.451	.742	63.7
Peking.....	95.6	.276	.564	48.4
Mandarin <sup>2</sup> .....	94.4	.507	.848	72.8
Morse.....	99.2	.346	.617	53.0
Average.....	96.2	.371	.668	57.8

<sup>1</sup> The coefficients of variability for duplicate or more determinations on the same variety (the number of separate determinations made on the same variety are indicated by the numbers in parentheses) were as follows: Illini (3) 2.5 percent, Ohio (3) 2.6, Harbinsoy (5) 6.2, Virginia (2) 3.7, Mansoy (3) 0.9, Ito San (3) 3.4, Dunfield (3) 5.7, Manchu (3) 2.7, Peking (3) 5.4, Mandarin (2) 5.1, and Morse (2) 0.86.

<sup>2</sup> The extraction procedure in this case was modified by using a 2-percent trichloroacetic acid extraction after the alcohol extraction and then precipitating the protein extracted with colloidal iron.

It may be noted from table 2 that the cystine determinations were made on extracts which contained on an average 96.2 percent of the total nitrogen in the whole beans. In only 2 of the 11 samples was there less than 93 percent of the total nitrogen in the extract.

Regardless of the manner in which the cystine is expressed, i. e., as a percent of the whole seed, as cystine nitrogen in percent of the total nitrogen, or as milligrams of cystine per gram of total nitrogen, there are large and significant differences. The percentage of cystine in the whole seed varied from 0.213 (Virginia) to 0.553 (Mansoy). The percentage of the total nitrogen which was cystine nitrogen varied from 0.385 (Virginia) to 1.042 (Mansoy), that of Mansoy being nearly 200 percent greater than that of Virginia. Expressed as milligrams of cystine per gram of total nitrogen, the variation was 33.1 (Virginia) to 89.4 (Mansoy).

Because of the completeness with which the total nitrogen of the beans was extracted, the cystine values reported here should repre-

sent more accurately than any heretofore published the actual amount of this amino acid present in the whole bean. The only previously reported results with which the results of this investigation may be compared, are those reported by Csonka and Jones (5), and even here the samples were not exactly comparable since Csonka and Jones analyzed a 10-percent sodium chloride extract (containing 85 to 90 percent of the total nitrogen) of the defatted meals. And of course difference in soils and environment might also add to any differences observed. Nevertheless, there were two varieties studied by both laboratories. Csonka and Jones reported 0.287 percent of cystine in defatted meal from Illini beans, while 0.451 percent of cystine may be calculated to have been present in the fat-free beans analyzed by the writers. Similarly, Csonka and Jones found 0.388 percent and the writers found 0.334 percent of cystine in the fat-free Peking variety. That there is probably a higher content of cystine in the glycinin fraction than there is in the entire mixed proteins of the soybean, is indicated by Csonka and Jones (4), who reported 0.74 and 1.45 percent cystine in the glycinin from the Illini and Manchu varieties, respectively, while the writers found but 0.366 and 0.451 percent of cystine in the whole beans of the same varieties.

While the results of the present study, as well as those of previous investigations, strongly indicate considerable varietal differences with respect to the cystine content of soybeans, the effects of other factors should not be overlooked. The same varieties grown in a single location may also vary considerably from year to year. O'Kelly and Gieger (18) found, over a 7-year period, variations in the protein content between 35.55 and 40.67 percent for the Loreda beans and between 39.91 and 44.64 percent for the Mammoth Yellow variety. That other environmental conditions, such as rainfall, fertility of soil, and temperature, may cause large differences in the protein content of the same variety has been shown by Webster and Kiltz (26). These investigators reported the protein content of four varieties of soybeans grown in two different counties of Oklahoma. Considerable variation was found in all varieties, but by far the greatest was that exhibited by the Loreda beans that contained 35.00 percent of protein when grown in Craig County and 47.50 percent when grown in Payne County (at Stillwater).

Thus, while wide differences observed in this study in the cystine content of different varieties of soybeans probably represent true varietal differences, the above-mentioned studies, indicating that environmental factors may cause wide differences in the protein content of the same variety, suggest the probability that the small differences observed in the cystine content of different varieties may not be true varietal differences.

Since the mixed proteins, rather than isolated proteins, are used most often as human and animal foods, these values on the cystine content of several common varieties of soybeans should be of interest. Also since cystine has been shown to be the limiting amino acid in the utilization of certain soybeans, a knowledge of the varietal variation should be of value to all interested in using soybeans as a protein supplement. It is conceivable, on the basis of their cystine content, that certain varieties would find their greatest value for feeding purposes while other varieties may be grown for industrial

purposes. For those varieties in which cystine has been shown to be the limiting factor in the utilization of their protein, it is obvious (27) that methionine also must be present in insufficient amounts to satisfy the combined requirements for both sulfur-containing amino acids.

Although Baernstein (1) reported the presence of 1.84 percent of methionine in glycinin from soybeans, little is known concerning the variation of this amino acid in different varieties of whole soybeans. It is, however, reasonable to suppose that there exists among different varieties a variation of considerable magnitude in the methionine content.

The inadequacy of a chemical estimation of a food nutrient in satisfying the animal's requirements may be emphasized in this connection. Compared with other foods and feeds, soybeans in general are not particularly low in their content of sulfur-containing amino acids, as indicated by chemical analyses. Nevertheless the sulfur-containing amino acids limit the utilization of raw soybean protein in growing animals. Heating the soybeans apparently makes the cystine or its equivalent methionine more available so that the nutritive value of the heated protein is approximately equal to that of the raw protein supplemented with cystine (8).

#### SUMMARY AND CONCLUSIONS

The cystine content of extracts, containing on an average 96.2 percent of the total nitrogen content of 11 varieties of soybeans all grown in the same year on uniform soil, was determined. Expressed as a percent of the whole soybean seed, the cystine content varied from 0.213 for Virginia beans to 0.553 for Mansoy beans. Expressed in milligrams of cystine per gram of nitrogen, a variation from 33.1 for the Virginia beans to 89.4 for the Mansoy variety was found. These latter figures show nearly 200 percent variation, much of which probably represents true varietal differences.

Since cystine or its equivalent in nutrition has been shown to be the limiting factor in the utilization of at least certain varieties of soybeans, and since there may be large differences in their cystine content, it is concluded that perhaps certain varieties should find their greatest value as protein supplements in human diets and in animal rations while others should find their greatest usefulness in the industries.

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